Epigenetics 8:5, 1–8; May 2013; © 2013 Landes Bioscience

First evidence of DNA methylation in insect *Tribolium castaneum*

Environmental regulation of DNA methylation within heterochromatin

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Keywords: DNA methylation, heterochromatin, satellite DNA, heat shock response, *Tribolium castaneum*

DNA methylation has been studied in many eukaryotic organisms, in particular vertebrates, and was implicated in developmental and phenotypic variations. Little is known about the role of DNA methylation in invertebrates, although insects are considered as excellent models for studying the evolution of DNA methylation. In the red flour beetle, *Tribolium castaneum* (Tenebrionidae, Coleoptera), no evidence of DNA methylation has been found till now. In this paper, a cytosine methylation in *Tribolium castaneum* embryos was detected by methylation sensitive restriction endonucleases and immuno-dot blot assay. DNA methylation in embryos is followed by a global demethylation in larvae, pupae and adults. DNA demethylation seems to proceed actively through 5-hydroxymethylcytosine, most probably by the action of TET enzyme. Bisulfite sequencing of a highly abundant satellite DNA located in pericentromeric heterochromatin revealed similar profile of cytosine methylation in adults and embryos. Cytosine methylation was not only restricted to CpG sites but was found at CpA, CpT and CpC sites. In addition, complete cytosine demethylation of heterochromatic satellite DNA was induced by heat stress. The results reveal existence of DNA methylation cycling in *T. castaneum* ranging from strong overall cytosine methylation in embryos to a weak DNA methylation in other developmental stages. Nevertheless, DNA methylation is preserved within heterochromatin during development, indicating its role in heterochromatin formation and maintenance. It is, however, strongly affected by heat stress, suggesting a role for DNA methylation in heterochromatin structure modulation during heat stress response.

Introduction

Methylation of cytosine is widely spread in eukaryotes and seems to be important for transcriptional regulation, heterochromatin formation and transposon inactivation.[1] In mammals, cytosine methylation represents the epigenetic mark responsible for genomic imprinting.[2] DNA methylation occurs in vertebrates predominantly at CpG sites and approximately 60% to 90% of CpG dinucleotides are methylated globally throughout the genome.[3] Presence of 5-methylcytosine has been reported in several insects (for a review see reference [4]), but the number of methylated CpGs is significantly lower than in vertebrates, as revealed by the whole genome methylation analysis of the silk worm *Bombyx mori*,[5] and honey bee *Apis mellifera*.[6] In the honey bee *A. mellifera*, DNA methylation has been implicated in the regulation of phenotypic plasticity and seems to determine the developmental fates in response to environmental signals.[7] However, further research is required to fully understand the role of DNA methylation in this evolutionarily diverse class.[8,9]

The beetle *Tribolium castaneum* is an important pest of stored products and a powerful model organism for the study of insect development. Until now, no evidence of genomic methylation in *T. castaneum* adults was demonstrated.[10] In addition to *T. castaneum,* a near-total lack of DNA methylation was reported in the fruit fly *Drosophila melanogaster* as well as in mosquito *Anopheles gambiae*.[11] The currently available genome sequence of *T. castaneum* seems to encode for DNA methyltransferases DNMT1 and DNMT2 and for MBD (methyl-CpG-binding domain) proteins.[12] DNMT1 and DNMT3 are generally considered necessary to a functional DNA methylation system and the absence of DNMT3 in *T. castaneum* is proposed to be associated with the loss of DNA methylation.[13] Intriguingly, the same set of DNMTs as in *T. castaneum* is found in the silk worm *B. mori,* but this species exhibits genomic methylation, although at low level.[5]

In insects, DNA methylation seems to be specific for intragenic regions while transposons and other intergenic repetitive elements are largely unmethylated.[3,10,14] However, there are examples of strongly methylated interspersed repetitive DNA

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Submitted: 02/27/2013; Revised: 03/26/2013; Accepted: 04/01/2013
http://dx.doi.org/10.4161/epi.24507